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Molecular characterization of *Fusarium* head blight resistance from wheat variety Wangshuibai

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Summary

Fusarium head blight (FHB) is a destructive disease of wheat worldwide. FHB resistance genes from Sumai 3 and its derivatives such as Ning 7840 have been well characterized through molecular mapping. In this study, resistance genes in Wangshuibai, a Chinese landrace with high and stable FHB resistance, were analyzed through molecular mapping. A population of 104 F_2-derived F_7 recombinant inbred lines (RILs) was developed from the cross between resistant landrace Wangshuibai and susceptible variety Alondra's'. A total of 32 informative amplified fragment length polymorphism (AFLP) primer pairs (*Eco*RI/*Mse*I) amplified 410 AFLP markers segregating among the RILs. Among them, 250 markers were mapped in 23 linkage groups covering a genetic distance of 2,430 cM. In addition, 90 simple sequence repeat (SSR) markers were integrated into the AFLP map. Fifteen markers associated with three quantitative trait loci (QTL) for FHB resistance ($P < 0.01$) were located on two chromosomes. One QTL was mapped on 1B and two others were mapped on 3B. One QTL on 3BS showed a major effect and explained up to 23.8% of the phenotypic variation for type II FHB resistance.

Abbreviations: cM: centimorgan; LOD: log likelihood ratio; RILs: recombinant inbred lines; AFLP: amplified fragment length polymorphisms; FHB: *Fusarium* head blight; SSR: simple sequence repeat

Introduction

Fusarium head blight (FHB), caused by *Fusarium gramineraum*, is a destructive disease of wheat and barley in warm and humid regions worldwide. In China, it was first reported in the late 1930's (Dai, 1941) and is becoming more frequent, severe and widespread (Chen et al., 2000). FHB causes severe yield loss and significantly lowers grain quality. In severe epidemics, disease incidence can reach up to 70% in susceptible varieties (Chen et al., 2000). In addition, infected grain is contaminated with mycotoxins such as deoxynivalenol that are toxic to animals and humans (Bai & Shaner, 1994; McMullen et al., 1997). Breeding wheat cultivars resistant to FHB is the preferable approach to minimize FHB damage. However, progress in breeding FHB resistant cultivars has been hindered by the lack of effective resistance sources and by the complex nature of wheat resistance. Furthermore, selection based on visual symptoms may not be effective because of confounding environmental effects such as temperature and humidity at flowering on expression of resistance genes.

Sumai 3 and its derivatives such as Ning 7840 have been used extensively as sources of resistance for wheat breeding programs worldwide (Bai & Shaner, 1994; Kolb et al., 2001). Resistance genes from Sumai 3 and related lines have been well characterized through molecular mapping (Bai et al., 1999; Waldron et al., 1999; Zhou et al., 2002). Molecular mapping using restriction fragment length polymorphism (RFLP) showed that three genomic regions in Sumai 3 were significantly associated with the FHB resistance (Waldron et al., 1999). The quantitative trait locus (QTL) on chromosome 3B of Sumai 3 demonstrated a major effect on FHB resistance and has been validated in several other populations (Buerstmayr et al., 2002; Anderson et al., 2001; Zhou et al., 2002). Exploration of new genetic sources other than Sumai 3 is still necessary to enhance genetic diversity of FHB resistance. Wangshuibai is a Chinese landrace and has been proved to possess a high level of resistance to fungal spread within a spike (type II resistance, Schroeder & Christensen, 1963). Repeated evaluations of FHB resistance under multi-environments in China have shown that FHB resistance in Wangshuibai is more stable than that of Sumai 3 (Lu et al., 2001). Thus, exploration of FHB resistance in Wangshuibai may provide breeders with alternative genes for the improvement of FHB resistance in wheat. The objectives of this study were to characterize QTL for FHB resistance in Wangshuibai with molecular markers and identify markers closely linked to FHB resistance for marker-assisted selection.

Materials and methods

Plant materials

A population of 104 F_2-derived F_7 RILs was developed by single-seed-descent from the cross Wangshuibai/Alondra's'. Wangshuibai is a highly FHB-resistant Chinese landrace from Jiangsu Province while Alondra's' is a highly susceptible variety from the International Maize and Wheat Improvement Centre (CIMMYT) carrying the 1RS. 1BL translocation chromosome.

Evaluation of FHB resistance

Both parents and their 104 F7 RILs were evaluated for FHB resistance at the Research Farm of Jiangsu Academy of Agricultural Sciences, Nanjing, China in 1998 and 2000, and the Research Farm of Huazhong Agriculture University, Wuhan, 2000. In each field experiment, 10 to 15 plants per line were inoculated and evaluated for type II resistance. About 10 μl conidiospore suspension (100 conidia per μl) of *F. gramineraum* was injected into a central spikelet of a spike at early anthesis (Lu et al., 2000). Inoculated spikes were covered with a plastic bag for three days to meet the moisture requirement for fungal infection. Percentage of scabbed spikelets was calculated as FHB severity at the 21st day after inoculation. The same materials were also evaluated for type II FHB resistance in a greenhouse at Oklahoma State University, Stillwater, OK, U.S.A., in 2001 and 2002. The same inoculation method was used for the greenhouse FHB evaluation except that inoculated plants were placed in a moist chamber for 72 hr to maintain moisture for initial fungal infection.

Molecular marker analysis

DNA from parents and 104 F7 RILs was extracted from young leaves using the CTAB method (Saghai-Maroof et al., 1984) with minor modifications. AFLP analysis (Vos et al., 1995) was conducted at Oklahoma State University according to Bai et al. (1999). In brief, about 500 ng genomic DNA was double-digested with the restriction enzymes *Eco*RI and *Mse*I. Digested DNA fragments were ligated with corresponding adapters and pre-amplified with non-selective primers. The corresponding *Eco*RI primer was labeled with ^{33}P-γ ATP. The selective bases on the 3′ prime end of primers for AFLP amplification were: AAC, AAG, ACA, ACAG, ACC, ACT, AGTG, AGC, AGT, GTG, and TCG for *Eco*RI primers and CAT, CTA, CTC, CTG, CTGA, CTT, GCG, and TGC for *Mse*I primers. PCR was conducted in a MJ Research PTC-100 Thermal Cycler. PCR products were separated in 5% polyacrymide gels and visualized by exposing the dried gel to a Kodak Biomax MR X-ray film.

Wheat SSR primers were synthesized according to the sequences described by Röder et al. (1998) and the United States Wheat and Barley Scab Initiative (website: www.scabusa.org). PCR amplification was performed according to Röder et al. (1998) in a Perkin-Elmer 9600 Thermal Cycler. The amplified products were separated in 6% polyacrylamide gels visualized by silver staining or in 3% agarose gels stained by ethidium bromide.

Linkage analysis

An AFLP linkage map was constructed using Mapmaker Program with Kosambi mapping function (Macintosh version 2.0, Lander et al., 1987). Threshold of log likelihood ratio (LOD) was set at 6.0. An integrated SSR and AFLP map was constructed with JoinMap® 3.0 Program (van Ooijen & Voorips, 2001). Regression analysis was used to calculate single marker associations with FHB resistance. Interval mapping analysis was carried out for further QTL analysis at LOD threshold of 2.5 with cofactor selection using MapQTL 4.0 (van Ooijen et al., 2001).

Results

FHB resistance

Due to variation in environmental factors such as temperature and moisture during fungal infection, significant variation in FHB severity of each line was observed among testing years or testing locations ($P < 0.001$). The FHB severity data obtained from the different experiments were only moderately correlated. However, the frequency distributions of FHB severity for the population were similar among different years and locations. Mean percentage of scabbed spikelets of individual RILs ranged from 5.6% to 95.7% and demonstrated a continuous distribution (Figure 1).

AFLP map and markers linked to resistance

Of the 207 AFLP (*Eco*RI/*Mse*I) primer combinations screened, 167 primer pairs amplified scorable bands from parental DNA of Wangshuibai and Alondra's'. On average, each primer amplified eight polymorphic bands. For mapping, 32 informative primer pairs were used and amplified 410 segregating AFLP markers. Using MapMaker Program, an AFLP linkage map was constructed with 250 AFLP markers covering a genetic distance of 2,430 cM composed of 23 linkage groups (results not shown).

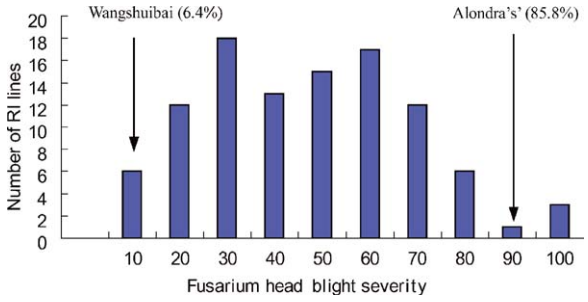

Figure 1. Frequency distribution of disease severity in RI lines of Wangshuibai/Alondra's'. Data based on average of five experiments.

Regression analysis indicated that 11 AFLP markers were significantly associated with FHB resistance ($P < 0.005$) in at least one of the experiments (results not shown).

Chromosomal location of QTL for FHB resistance

To identify the chromosomal locations of individual linkage groups in the AFLP map, 302 SSR primer pairs from 21 chromosomes were screened and 103 primer pairs showing polymorphism between parents were selected to analyze the population, of which 90 SSR markers were integrated into the AFLP map using JoinMap program. Based on known locations of SSR primers, putative QTL were mapped to chromosomes 1B and 3B. A total of five SSR markers and four AFLP markers mapping to chromosome 3BS were associated with FHB resistance. The SSR loci *Xgwm161* and *Xgwm285* explained 11.8% and 7.3% of phenotypic variation for FHB resistance, respectively, using the phenotypic data from the greenhouse experiment (data not shown). These two SSRs were mapped to different positions on 3BS chromosome (Figure 2). Two QTL were thus detected on chromosome 3BS in the intervals of *Xbarc147* to *Xgwm493* and *Xgwm285* to *XEtcgMctc11*, respectively. In addition, one putative QTL was identified in the genomic interval between *XEtcgMagc7* and *XEactgMctc7* on chromosome 1B in the experiment, Wuhan 2000 (Figure 2).

Interval mapping based on the data from USA2001 and USA2002 revealed that the QTL on chromosome 3B is located between *Xbarc147* and *Xgwm493* and explained 13.7% and 23.8% of the phenotypic variation, respectively (Table 1). The other QTL on chromosome 3BS mapped between *Xgwm285* and *XEtcgMctc11* with LOD values of 1.69 and 3.43, respectively. These two QTL were contributed by parent Wangshuibai. Interval mapping based on the data from WH2000 also revealed a putative QTL for FHB resistance on chromosome 1BS contributed by Alondra's'. Since Alondra's' carries the 1RS.1BL translocation chromosome, this QTL in chromosome 1B is in reality located in chromosome 1RS. It explained 15.6% of the phenotypic variation (Table 1).

Table 1. Marker interval, chromosome location and determination coefficient of QTL for FHB resistance

Map interval	Chromosome	Origin[a]	Data set	LOD	$R^2 \times 100$	Additive effect
Xbarc147 – Xgwm493	3B	W	USA2001	2.82	13.7	7.69
			USA2002	4.58	23.8	9.17
Xgwm285 – XEtcg.Mctc-11	3B	W	USA2001	1.69	7.1	5.61
			USA2002	3.43	15.7	8.34
XEtcg.Magc-7 – XEaccg.Mctc-7	1B	A	WH2000	2.71	15.6	−7.16

[a]W = Wanghuibai; A = Alondra's'.

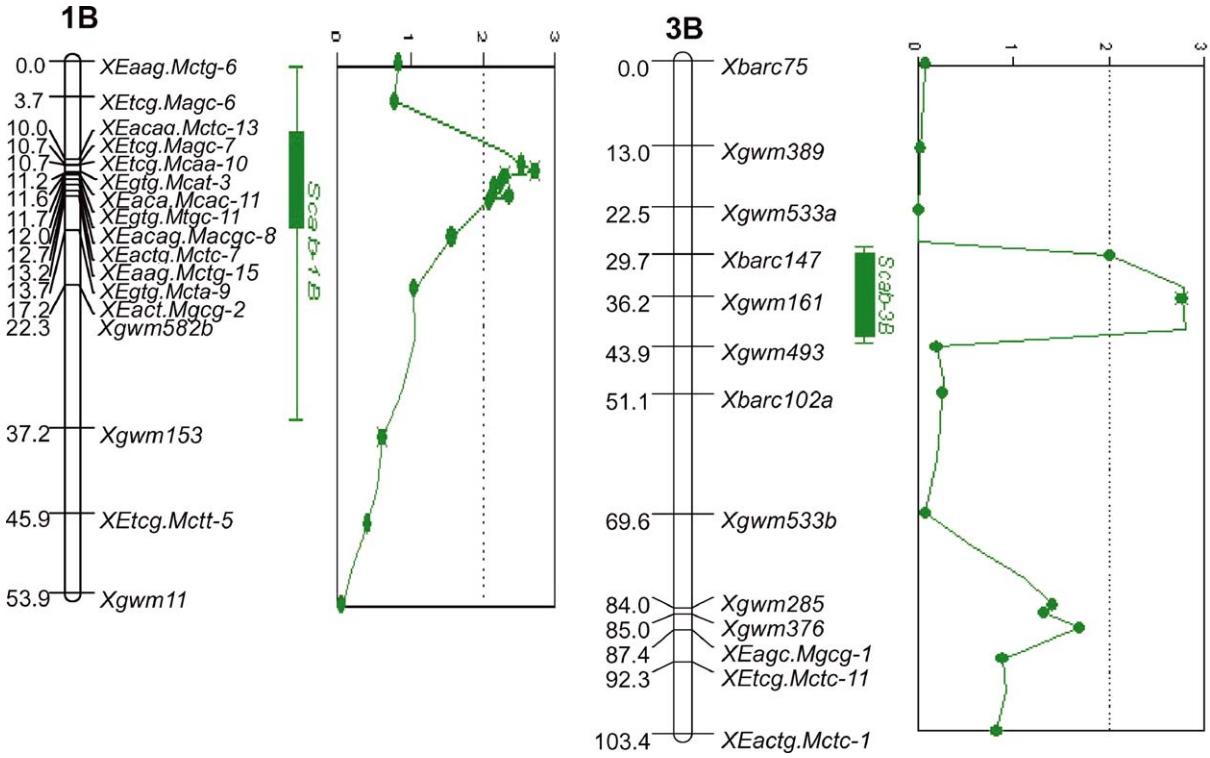

Figure 2. Putative QTL on chromosome 1B based on data WH2000 and on chromosome 3B based on data USA2001.

Discussion

Previous works indicated that resistance to wheat FHB is a quantitative trait and greatly affected by environmental factors (reviewed by Kolb et al., 2001; Lu et al., 2001). Accuracy in phenotyping of FHB resistance directly affects the power of QTL detection. In this study, correlation of FHB severity of RILs among experiments was not as high as we expected (Table 2) although appropriate moisture was provided for initial fungal infection either by covering each inoculated spike with a plastic bag for three days or enclosing inoculated plants in a moist chamber. The largest effects

Table 2. Correlation coefficients for disease severity among five experiments

Correlation coefficient	NJ2000	WH2000	USA2001	USA2002
NJ1998[a]	0.5233	0.4074	0.4857	0.5120
NJ2000		0.6088	0.3478	0.5317
WH2000			0.4345	0.2190
USA2001				0.5417

[a]NJ1998, NJ2000, WH2000, USA2001 and USA2002 represent that FHB were tested in 1998 and 2000 in Nanjing, 2000 in Wuhan and 2001 and 2002 in Oklahoma, U.S.A., respectively.

were detected for two QTLs on chromosome 3B when FHB data from greenhouse experiments (Oklahoma, 2001, 2002) were used for QTL analysis. The other three experiments were conducted under field conditions where temperature and moisture may be more variable than under controlled greenhouse conditions. Therefore, it is recommended that evaluation of Type II FHB resistance should be carried out in the greenhouse in order to obtain more accurate assessments than in the field.

Wangshuibai was repeatedly reported to possess higher and more stable resistance to FHB than many other sources (Bai & Shaner, 1994; Lu et al., 2001). Our results suggested that FHB resistance in Wangshuibai is likely to be controlled by a minimum of three genes, which is in agreement with reports by Bai et al. (1990) and Lin et al. (1992). But our results contradict those of Liao et al. (1985) who suggested genes on chromosomes 4A, 5A, 7A, 7B, 4D and 2D based on monosomic analysis. The difference may be due to several factors including disease evaluation method, genetic background effects from the susceptible parents, or the interaction between genotype and environment.

Two genomic regions on chromosome 3BS were associated with FHB resistance. It is assumed that the two significant regions on the same chromosome are two independent QTL because they are separated by a map distance of more than 20 cm. Furthermore, interval mapping indicated there were two QTLs.

It has been reported that Sumai 3, a well-known Chinese FHB resistance resource, possesses a major QTL on chromosome 3BS (Waldron et al., 1999; Anderson et al., 2001; Buerstmayr et al., 2002). This QTL was placed in the map interval *Xgwm533.1* to *Xgwm493* and explained about 42% of the phenotypic variation for FHB resistance in Sumai 3, and was designated *Qfhs.ndsu-3BS* (Anderson et al., 2001). Shen et al. (2003) found that a major QTL in Ning 894037, another Chinese FHB resistance resource, was also in the same genomic region on 3BS. This QTL explained 42% of the phenotypic variation. Similar results were reported by Zhou et al. (2002), Buerstmayr et al. (2002, 2003), Bourdoncle & Ohm (2003) & Somers et al. (2003). Results from the present study indicated that the FHB resistance QTL with significant effect from Wangshuibai was located on chromosome 3BS and flanked by markers *Xbarc147* and *Xgwm493*. This QTL explained up to 23.8% of the phenotypic variation for FHB resistance. The FHB resistance effect of the QTL in Wangshuibai seems to be smaller than the one in Sumai 3 and related sources (Bai et al., 1999; Zhou et al., 2002). It is possible that a resistance gene cluster exists on chromosome 3B with different alleles at linked loci. It is also possible that these 3BS QTL from different sources are indeed the same QTL. The difference in QTL effects reported in the different resistant varieties and mapping studies might be due to the size of the mapping populations, the accuracy and methods of the resistance evaluations used in the different studies and the interaction of the 3BS QTL with different genetic backgrounds. Wangshuibai is a Chinese landrace, Sumai 3 was derived from a cross between the Italian variety Funo and the Chinese landrace Taiwan Wheat, while Ning 894037 was selected from a somaclonal variant of the FHB susceptible variety Yangmai 3 (Shen et al., 2003). Bai et al. (2003) showed that the markers associated with FHB resistance of Sumai 3 were derived from Taiwan Wheat. Liu & Anderson (2003) reported that Wangshuibai has a haplotype different from Sumai 3 at five SSR marker loci near *Qfhs.ndsu-3BS*. Further research is needed to clarify the relationship between FHB-resistance QTL on 3BS mapped in different FHB resistance sources.

In this study, three FHB resistance QTL in the Chinese landrace Wangshuibai were identified, providing more FHB resistance resources to improve wheat resistance to FHB by gene pyramiding. Although the major QTL on chromosome 3BS has already been identified from other sources, the two smaller QTLs on chromosomes 1R and 3B are new. Marker-assisted selection (MAS) for FHB resistance genotypes can be performed using some of the closely linked markers, especially breeder-friendly SSR markers. Furthermore, markers associated with agronomic traits such as plant height and heading date can also be identified in the AFLP-SSR integrated map and can be used for MAS to break undesired associations between FHB resistance and other agronomic traits.

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